Evaluation of Array Comparative genomic Hybridisation in prenatal diagnosis of fetal anomalies: a multicentre cohort study with cost analysis and assessment of patient, health professional and commissioner preferences for array comparative genomic hybridisation

Stephen C Robson,[1]* Lyn S Chitty,[2] Stephen Morris,[3] Talitha Verhoef,[3] Gareth Ambler,[4] Diana G Wellesley,[5] Ruth Graham,[6] Claire Leader,[1] Jane Fisher[7] and John A Crolla[8]

[1]Institute of Cellular Medicine, Newcastle University, Newcastle, UK
[2]Institute of Child Health, University College London, London, UK
[3]Department of Applied Health Research, University College London, London, UK
[4]Department of Statistical Science, University College London, London, UK
[5]Department of Genetics, Princess Anne Hospital, Southampton, UK
[6]School of Geography, Politics and Sociology, Newcastle University, Newcastle, UK
[7]Antenatal Results and Choices, London, UK
[8]Wessex Regional Genetics Laboratory, Salisbury, UK

*Corresponding author

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Disclaimer: This report contains transcripts of interviews conducted in the course of the research and contains language that may offend some readers.

Scientific summary

The EACH Study

Scientific summary

Background

Current pathways for testing fetuses at increased risk of a chromosomal anomaly because of an ultrasound anomaly involve karyotyping after rapid aneuploidy exclusion by quantitative fluorescent polymerase chain reaction (QF-PCR). Chromosomal microarray (CMA) may detect more clinically significant chromosomal imbalances, but evidence to guide UK health service providers on whether or not CMA should replace karyotyping is limited.

Objectives

1. To compare detection rates of copy number variants (CNVs) and variants of unknown significance (VOUS) by karyotyping and CMA in two target populations of fetuses with anomalies detected on ultrasound and to compare laboratory turnaround times (TATs) from receipt of sample to issuing of results for karyotyping with CMA.
2. To calculate costs of karyotyping and CMA to NHS and Personal Social Services [including deoxyribonucleic acid (DNA) extraction, quality metrics, labelling and follow-on tests on fetal/parental DNA] and to calculate the cost per additional pathogenic CNV detected by array comparative genomic hybridisation (CGH) relative to karyotyping.
3. To evaluate parents' and health professionals' attitudes to CMA and to determine what factors influence their choice and decision-making about CMA (and the potential to replace karyotyping).
4. To contribute maternal blood samples to the National Institute for Health Research (NIHR)-funded Reliable Accurate Prenatal non-Invasive Diagnosis (RAPID) project (the methods and results of which will be reported separately).

Methods

The Evaluation of Array Comparative genomic Hybridisation (EACH) study was a multicentre experimental research cohort study with an additional cost analysis and qualitative substudy. The study population consisted of women with a fetus undergoing QF-PCR and conventional karyotyping by amniocentesis, chorionic villus sampling or fetal blood sampling for clinical indications with one or more structural anomalies identified on an ultrasound scan at any gestational age or an isolated nuchal translucency (NT) of ≥ 3.5 mm at 11^{+2} to 14^{+1} weeks of pregnancy. With the exception of fetuses with a nuchal fold of > 6 mm at 18^{+0} to 20^{+6} weeks of pregnancy, fetuses with single or multiple ultrasound variants (or markers) were excluded.

Eligible women attending 20 fetal medicine units in England and Wales gave written informed consent to use any excess fetal material available after setting up routine testing (QF-PCR and karyotyping) and donate a blood sample. When partners were in attendance they were also approached to donate a blood sample. Only fetuses with a normal QF-PCR result or a sex chromosome aneuploidy that was unlikely to explain the ultrasound anomaly (e.g. XXX, XXY and XYY underwent CMA).

All nine participating cytogenetic laboratories followed their existing clinical pathways for karyotyping and adapted their workflows to incorporate DNA extraction from amniocytes and chorionic villi for both QF-PCR and CMA. The same oligonucleotide-CGH array design, consisting of an 8-plex of 60,000 60-mer oligonucleotides with a backbone resolution of ≈ 75 kb but with considerably higher coverage over chromosomal regions of interest, was used by all laboratories. CMA results were analysed using the software supplied with the array and

presented in GRch37/hg19 format. Several laboratory techniques (including parental karyotyping, parent versus parent CMA and fluorescence in situ hybridisation) were used to determine the parental origins of CNVs detected by karyotyping or CMA.

For the purposes of the EACH study, all structural and numerical karyotype abnormalities, whether balanced or not, were included as abnormal karyotypes. Laboratories utilised their own standard operating procedures to classify CNVs detected by their analytical CMA software as (1) pathogenic, (2) VOUS (when the possible genotype and phenotypic effects were uncertain) or (3) benign and not relevant to the presenting phenotype. When local laboratories were uncertain about classifying a CNV, an expert review panel (comprising at least two clinical geneticists and two cytogeneticists) was available for advice. All pathogenic CNVs were reported to clinicians but VOUS were not reported. Clinical and laboratory data (including fetal ultrasound findings and postnatal outcome information) were recorded on a bespoke database (Cartagenia Bench™ version 4.0, Cartagenia N.V., Leuven, Belgium).

Turnaround times for conventional karyotyping and CMA were measured from the date the fetal sample was received in the laboratory to the date the final karyotype or CMA report was issued by the laboratory. Data on actual set-up to reporting times were also collected for each test.

A sample of 500 in each target population was selected to give in excess of 90% power to detect a difference in detection rates at the 5% significance level, assuming karyotyping- and CMA-detected anomalies in 5% and 10% of fetuses, respectively. This sample size would give a 95% confidence interval (CI) for the difference in detection rates of ±2%. The proportion of subjects with pathogenic CNVs and VOUS were calculated with exact 95% CIs. Statistical comparisons of pathogenic CNVs/VOUS between karyotyping and CMA were made using McNemar's test.

For the economic analysis, a decision-analytic model was developed describing the two diagnostic options (karyotyping and CMA) and the possible follow-on pathways using data collected on Cartagenia Bench. Data on the costs of karyotyping and CMA were collected from nine cytogenetic laboratories. The average costs were used as base-case values in the current model. The costs of clinical follow-up focused specifically on follow-up consultations related to test results. The costs of the EACH panel review were also calculated. All costs are expressed in 2012–13 Great British pounds. The time horizon in this study was the duration of pregnancy; therefore, no discounting was necessary. The main outcome of the model was the incremental costs per pathogenic CNV detected by CMA compared with karyotyping. Uncertainty around input parameters was taken into account using one-way sensitivity analysis and probabilistic sensitivity analysis.

The qualitative substudy used a purposive sampling approach, to enhance diversity of views represented within the sample. Women who had participated in the EACH trial at three study sites and who had agreed to be interviewed about their experience were contacted and interviewed a minimum of 3 months after completion of the pregnancy. Health professionals from two study sites were selected to ensure the inclusion of a range of professional viewpoints. National and local commissioners were identified via a convenience sample through a national contact. The aim was to undertake 15–20 interviews in both the parent and health professional groups. Data collection was via in-depth, semistructured interviews, and data analysis of anonymised transcripts was informed by a generative thematic approach using the software package ATLAS.ti (version 7.0, Cleverbridge AG, Cologne, Germany). Coded data were organised into key themes and subthemes.

Results

In total, 1718 probands were recruited between March 2012 and May 2014. A paternal blood sample was obtained in 1347 (78.4%) cases and 1460 (84.9%) women gave their consent to be contacted after the birth to arrange assessment of their infant. Source tissue for DNA extraction was chorionic villi in 55.8% of cases, amniotic fluid in 40.8% and other fetal tissue in 2.7%. After exclusions (including 509 cases with a

common chromosomal anomaly detected by QF-PCR) and test failures (CMA in 49 cases, karyotype in nine cases), 1123 cases were available for comparison; 494 in the increased NT group and 629 in the structural anomaly group. The latter group included 105 (16.7%) cases in which an anomaly was detected at 11–14 weeks' gestation (with or without increased NT) and 75 (11.9%) cases detected after 24 weeks' gestation. After a total of 268 follow-up tests (including 91 parent vs. parent arrays and 81 fluorescence in situ hybridisation studies), 15 (1.3%) cases were classified as abnormal karyotype and normal CMA, 58 (5.2%) as abnormal karyotype and pathogenic CNV on CMA, 42 (3.7%) as normal karyotype and pathogenic CNV on CMA and 38 (3.4%) as normal karyotype and VOUS on CMA. Within the increased NT group, 55 out of 307 (17.9%) with known pregnancy outcome opted for termination of pregnancy [including 24/62 (38.7%) with an abnormal karyotype with or without CMA] compared with 153 out of 462 (33.1%) in the structural anomaly group [including 46/88 (52.2%) with an abnormal karyotype with or without CMA].

In the increased NT group, there was no difference in the rates of abnormal karyotype and pathogenic CNV on CMA but the rate of any CNV (pathogenic + VOUS) on CMA was 4.5% (95% CI 1.8% to 7.1%) higher than the rate of abnormal karyotype. The rate of pathogenic CNVs on CMA was higher in fetuses with a NT of > 5 mm than in fetuses with a NT of 3.5–5 mm (13.2% vs. 5.4%; $p < 0.003$). In the structural anomaly group, CMA detected more CNVs (6.8%, 95% CI 4.4% to 9.3%) and more pathogenic CNVs (3.5%, 95% CI 1.5% to 5.5%) than karyotyping. The rate of pathogenic CNVs on CMA was similar in fetuses with multiple structural anomalies or one structural anomaly (11.1% vs. 8.8%; $p = 0.34$) but was higher in fetuses with a cardiac than a non-cardiac anomaly (14.5% vs. 7.9%; $p = 0.013$).

Median [interquartile range (IQR)] TATs were 12 days (IQR 10–14 days) for karyotyping and 15 days (IQR 12–25 days) for CMA (difference 3 days, IQR 0–13 days; $p < 0.0001$). However, when actual set-up to reporting times were compared, CMA was 5 days (IQR 2–8 days) quicker. Median set-up to reporting times varied significantly between laboratories. In total, 32 cases with CNVs on CMA were referred to the EACH review panel; 16 were classified as pathogenic and reported and 16 were classified as VOUS and not reported. Out of the 16 recommended for reporting, 9 (56%) were < 1 Mb in size and 6 out of 16 (38%) of those not reported were > 1 Mb.

The average cost of CMA was higher (£322, range £280–367) than the cost of karyotyping of chorionic villi (£272, range £240–307) and amniotic fluid (£234, range £212–258). Cost calculations of the respective pathways indicated that the per-patient CMA was, on average, £113 more costly than karyotyping. The incremental cost per extra pathogenic CNV detected by CMA was £4703 for the whole study group, £9418 in the increased NT group and £3635 in the structural anomaly group. In a one-way sensitivity analysis, the only parameters found to affect the results appreciably were the number of pathogenic results and the test costs. In the probabilistic sensitivity analysis, 0% of the simulations were found to be cost-effective if the willingness to pay per incremental anomaly found was £1000 and 87% of the simulations were found to be cost-effective if the willingness to pay was £10,000.

Out of the 98 individuals invited to participate in the qualitative evaluation, 48 respondents were interviewed [16 women (five with partners present), 21 health professionals and six commissioners]. Using generative thematic analysis, four key themes emerged from the data: (1) the functionality of CMA, (2) introducing CMA into the clinical consultation, (3) decision-making and (4) embedding CMA as standard clinical practice. Overall, the findings suggested that parents find CMA acceptable, despite the uncertainties it may introduce, on the basis that the test offers additional information. The majority of health professionals also found CMA acceptable and, along with commissioners, were supportive of its integration into clinical practice. The challenges of informed consent and managing uncertain prognosis were highlighted.

Conclusions

The EACH study was designed to provide guidance to health service providers on whether or not CMA should replace karyotyping in the prenatal diagnosis of fetal anomalies. The results suggest that CMA is a

robust, acceptable and probably cost-effective diagnostic test and should replace karyotyping in care pathways when the indication for fetal testing is one or more structural anomalies or an isolated NT of ≥ 3.5 mm on an ultrasound scan after a normal QF-PCR result. Replacement for both indications will ensure there is a consistent prenatal (and postnatal) diagnostic technology.

There is some evidence from the study that, until more national (and international) information on prenatal variants has been collected that specifically links clinical phenotypic and molecular data, there may be benefit in a national advisory group that can provide expert advice to local health-care professionals about variants of possible pathogenic significance. Such a group could also identify incidental findings not to be reported. The operating model of the EACH review panel could serve as a template for such a group. Furthermore, to ensure consistent high-quality information is provided to parents undergoing CMA, consideration should be given to producing a national information sheet and consent form.

Trial registration

This trial is registered as ISRCTN01058191.

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